

Status of the Claims

Claims 1-12 and 46 are pending. Applicants have amended claims 1-5, 10 and 11-12 and respectfully request entry of these amendments under 37 C.F.R. § 1.114. As amended, claim 1 recites "cycling cells sensitive to the effects of high energy electromagnetic radiation," "high energy electromagnetic radiation," and "synchronizing at least 30% of said cells." Support for this amendment can be found throughout the specification and claims as originally filed, for example, at page 9, lines 3-5, page 8, line 26-27, and page 18, line 21, respectively. As amended, claim 10 recites "wherein said nucleic acid is fully encapsulated." Support for this amendment can be found throughout the specification and claims as originally filed, for example, at page 26, lines 21-25. Claims 2-5 and 12 have been amended solely to ensure proper antecedent basis. Thus, no new matter has been introduced by these amendments.

A version of the claims with markings to show changes to the claims is provided in Appendix A. All of the pending claims are provided in Appendix B for the Examiner's convenience.

In the Advisory Action mailed November 7, 2002, the pending claims were rejected, in various combinations, under 35 U.S.C. § 112, second paragraph; 35 U.S.C. § 112, first paragraph; and under 35 U.S.C. § 103(a). Each of these rejections is addressed below.

Rejections Under 35 U.S.C. § 112, second paragraph

The Examiner alleges that "synchronizing at least 30% of said cells" raises issues under 35 U.S.C. § 112, second paragraph, because it is allegedly unclear how one would calculate or know the number of cells affected by the treatment. In addition, the Examiner alleges that the amendments which recite "high energy electromagnetic radiation" raise issues regarding new matter. Applicants respectfully traverse these rejections.

1. Allegation that the recitation "synchronizing at least 30% of said cells" is indefinite

As set forth in MPEP § 2173.02, "[d]efiniteness of claim language, must be analyzed in light of (A) content of the application; (B) the teachings of the prior art; and (C) the claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made."

In the instant case, the specification adequately defines the terms or the terms are adequately understood to one of skill in the art, such that the claims are not indefinite under 35 U.S.C. § 112, second paragraph. For example, the specification at page 6, lines 2-10; page 40, line 9

to page 41, line 6; and page 41 lines 26-28 provides ample detailed guidance for one of skill in the art to assess cell cycle status. In particular, the specification provides working examples that describe and demonstrate to one of skill in the art methods for determining the cell cycle status of cells (e.g., by flow cytometry of permeabilized cells stained with propidium iodide).

In view of the foregoing, Applicants respectfully submit that those of skill in the art could easily determine the number of cells affected by the treatment. Accordingly, Applicants urge the Examiner to withdraw the rejection under 35 U.S.C. § 112, second paragraph.

2. Allegation that the recitation "high energy electromagnetic radiation" introduces new matter

As set forth in MPEP § 2163.07(a), an application may be amended to recite an inherent property without introducing prohibited new matter. It is well known to those of skill in the art that there is a spectrum of electromagnetic radiation (*see, e.g.*, Serway, PHYSICS FOR SCIENTISTS & ENGINEERS, p. 386-387 (2nd ed. 1986), copy enclosed as Appendix A. Moreover, it is known that there are three types of electromagnetic radiation that are classified as high energy electromagnetic radiation, namely gamma rays, x-rays, and ultraviolet rays (*see, id.*). Thus, high energy is an inherent property of gamma rays, x-rays, and ultraviolet rays. Applicants respectfully note that each of these types of electromagnetic radiation are recited in the claims as originally filed. In addition, Applicants respectfully note that Bologna, *et al.* (provided with the Amendment filed October 10, 2000) discloses that high energy electromagnetic radiation includes ultraviolet rays. Therefore the amendment that recites "high energy electromagnetic radiation" is an amendment that recites an inherent property of gamma rays, x-rays, and ultraviolet rays and does not introduce new matter to the application. Therefore, Applicants respectfully request withdrawal of this aspect of the rejection.

Rejection Under 35 U.S.C. § 112, first paragraph

The claims are rejected under 35 U.S.C. § 112, first paragraph as allegedly nonenabled. Applicants respectfully traverse. As set forth in M.P.E.P. § 2164:

[a]ny analysis of whether a particular claim is supported by the disclosure in an application requires determination of whether that disclosure, when filed, contained sufficient information regarding the subject matter of the claims as to enable one skilled in the pertinent art to make and use the claimed invention. *** ('The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue

experimentation.'). A patent need not teach, and preferably omits, what is well known in the art. *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991), *Hybridtech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986).

1. Allegation that synchronizing at least 30% of cells is not enabled

In the Office Action mailed November 7, 2002, the Examiner alleges that the recitation "synchronizing at least 30% of said cells" raises issues under 35 U.S.C. § 112, first paragraph regarding the necessary teaching to achieve this specific level of synchronization. As explained above, the specification provides working examples that describe and demonstrate synchronization of cells. For example, the specification at page 6, lines 2-10; page 40, line 9 to page 41, line 6; and page 41 lines 26-28 provides ample detailed guidance for one of skill in the art to assess cell cycle status. In addition, the references provided with the Amendment filed October 10, 2000 demonstrate that synchronizing cells with high energy electromagnetic radiation was known to those of skill in the art at the time of filing the present application (*see, e.g.*, Rubin *et al.* (1988); Bolognia, *et al.* (1994); and Pellegata, *et al.* (1996)). Therefore, Applicants respectfully request withdrawal of this aspect of the rejection under 35 U.S.C. § 112, first paragraph.

2. Allegation that synchronization of cells using electromagnetic radiation is not enabled

In the Office Action mailed November 7, 2002, the Examiner acknowledges that Applicants arguments regarding the ability of various spectrums of electromagnetic radiation to synchronize cells at different cell cycle stages are persuasive, but states that since the claim amendments were not entered, the claims still encompass non-enabled embodiments. Applicants respectfully request entry of the amendments under 37 C.F.R. § 1.114 and request reconsideration of the arguments in light of the amendments.

As explained previously, Applicants amended claim 1 in accordance with the Examiner's suggestions in the Office Actions mailed January 4, 2001, and April 24, 2002 in order to expedite prosecution. Specifically, claim 1 has been amended to recite "cycling cells sensitive to the effects of high energy electromagnetic radiation" and "high energy electromagnetic radiation."

The Examiner previously alleged that the claims were not enabled because the specification allegedly lacks guidance or working examples that demonstrate that the cell can be synchronized in any other stage than G2/M. However, "[c]ompliance with the enablement

requirement of 35 U.S.C. § 112 first paragraph does not turn on whether an example is disclosed." (See, M.P.E.P. § 2164.02). Moreover, Applicants respectfully assert that it was known to those of skill in the art at the time the application was filed that electromagnetic radiation synchronizes cells at stages other than G2/M. For example, Rubin *et al.* (provided with the Amendment filed October 10, 2000) discloses that ultraviolet radiation synchronizes cells at either the G1 stage or the G1/S stage. Furthermore, Pellegata, *et al.* (provided with the Amendment filed October 10, 2000) discloses that gamma radiation synchronizes cells at either the G1/S stage or the G2/M stage. Thus, contrary to the Examiner's previous allegation, the same form of radiation does have the ability to synchronize cells at different stages in the cell cycle.

In view of the foregoing, Applicants respectfully submit that the claims are fully enabled. Accordingly, Applicants urge the Examiner to withdraw the rejection under 35 U.S.C. § 112, first paragraph.

Rejections Under 35 U.S.C. § 103

Claims 1-12 and 46 were previously rejected, in various combinations, under 35 U.S.C. § 103(a) over a number of different references. Applicants traversed these rejections, arguing that the cited references alone and in combination did not teach or suggest using electromagnetic radiation to synchronize at least 30% of cycling cells to improve transfection efficiency as disclosed and claimed in the present invention. In the Office Action mailed November 7, 2002, the Examiner states that the claims remain rejected because the arguments made to overcome the cited references encompassed claims amendments which had not been entered. Applicants respectfully request entry of the amendments under 37 C.F.R. § 1.114 and request reconsideration of the arguments in light of the amendments.

Applicants respectfully traverse each of the § 103 obviousness rejections. As set forth in M.P.E.P. § 2143:

[t]o establish a *prima facie* case of obviousness, three basic criteria must be met. *First*, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. *Second*, there must be a reasonable expectation of success. *Finally*, the prior art reference (or references when combined) must teach or suggest all the claim elements. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both

be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

All three elements set forth above must be present in order to establish a *prima facie* case of obviousness. As explained herein below in connection with each of the § 103(a) obviousness rejections, Applicants assert that a *prima facie* case of obviousness has not been established for at least the following reason: the cited references do not teach or suggest all of the claim limitations.

1. Rejection of claims 1-9, 11-12 and 46 over Yorifuji *et al.* in view of Spang-Thomsen *et al.*

Claims 1-9, 11-12, and 46 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Yorifuji *et al.* in view of Spang-Thomsen *et al.* In making this rejection, the Examiner previously alleged that Yorifuji *et al.* "demonstrate that through use of chemicals and electromagnetic radiation that synchronized cells are more efficiently transformed at different parts of the cell cycle," and that Spang-Thomsen *et al.* "teach the synchronization of *in vivo* analyzing various conditions for the optimization of synchronizing conditions." Applicants respectfully traverse.

As explained previously, Applicants respectfully submit that the teachings of Yorifuji *et al.* and Spang-Thomsen *et al.* have been mischaracterized. Yorifuji *et al.* disclose only that cells synchronized by two specific **chemicals**, hydroxyurea and aphidicolin, exhibit greater transformation efficiency when transformed with linearized plasmid DNA via electroporation (*see*, page 201, col.

2). There is no hint or suggestion in Yorifuji *et al.* of using electromagnetic radiation to synchronize cells. Spang-Thomsen *et al.* is focused on the use of radiotherapy to induce tumor cell death, *i.e.*, the effect of x-irradiation on the proliferation kinetics of malignant melanoma cells. Spang-Thomsen *et al.* disclose that exposure to x-rays induces **partial** synchronization of small fractions of malignant melanoma cells (*see*, abstract). Specifically, Spang-Thomsen *et al.* disclose that a maximum of 20% of malignant melanoma cells accumulated in the G2/M phases after exposure to x-rays, regardless of the dose or length of time post exposure to x-rays (*see*, Figure 1). According to Spang-Thomsen *et al.*, "[i]t is questionable whether cell accumulation of this magnitude could be utilized in the design of fractionated radiotherapy to increase the treatment effect" (*see*, page 852, col. 2). There is no hint or suggestion in Spang-Thomsen *et al.* of transfecting cells after exposure to x-rays, or of increased transfection efficiency of cells after exposure to x-rays. Thus, one of skill in the art would not have been motivated to combine Yorifuji *et al.* with Spang-Thomsen *et al.* because Spang-Thomsen *et al.*

teach away from using electromagnetic radiation to synchronize cycling cells in order to improve transfection efficiency as disclosed and claimed in the present invention. Moreover, even if the disclosures of Yorifuji *et al.* and Spang-Thomsen *et al.* were combined, they would not lead to the claimed invention because in contrast to Spang-Thomsen *et al.*, claim 1 recites "synchronizing *at least* 30% of said cells" (emphasis added).

Absent a teaching or suggestion to use electromagnetic radiation to synchronize at least 30% of cycling cells to improve transfection efficiency as disclosed and claimed in the present invention, the present invention is non-obvious and, thus, patentable. Accordingly, Applicants urge the Examiner to withdraw this rejection under 35 U.S.C. § 103(a).

2. Rejection of claim 10 over Yorifuji *et al.* in view of Spang-Thomsen *et al.*, further in view of Son *et al.*

Claim 10 stands rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Yorifuji *et al.* in view of Spang-Thomsen *et al.* as applied to claims 1-9, 11-12, and 46, and further in view of Son *et al.* In making the rejection, the Examiner previously alleged that Son *et al.* teach specifically how to transform a cell with a lipid-nucleic acid particle. Applicants respectfully traverse.

As explained above, one of skill in the art would not have had motivation to combine the teachings of Yorifuji *et al.* and Spang-Thomsen *et al.* Even if one of skill in the art were to combine Yorifuji *et al.* with Spang-Thomsen *et al.*, the combination would not lead to the claimed invention because Spang-Thomsen *et al.* teach away from using electromagnetic radiation to synchronize cycling cells to improve transfection efficiency as disclosed and claimed in the present invention. Son *et al.* do not remedy the deficiencies of Yorifuji *et al.* and Spang-Thomsen *et al.*

Son *et al.* disclose a DNA-liposome **complex** prepared by mixing DNA with liposomes (*see*, page 12669, col. 2). In the DNA-liposome complex of Son *et al.* the DNA is *not* fully encapsulated in the liposome. In contrast, to Son *et al.*, claim 10 recites "wherein said nucleic acid is fully encapsulated in a lipid-nucleic acid particle." The specification at page 26, lines 21-25 describes the encapsulated, nuclease resistant lipid-DNA particles as claimed in the present invention, and clearly they are different from the DNA-liposome complex of Son *et al.*

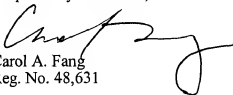
Absent a teaching or suggestion to use electromagnetic radiation to synchronize at least 30% of cycling cells to improve transfection efficiency of a nucleic acid fully encapsulated in a lipid nucleic acid particle, as disclosed and claimed in the present invention, the present invention is

non-obvious and, thus, patentable. Accordingly, Applicants urge the Examiner to withdraw this rejection under 35 U.S.C. § 103(a).

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is urged. If the Examiner believes a telephone conference would aid in the prosecution of this case in any way, the Examiner is invited to telephone the undersigned at 415-576-0200.

Respectfully submitted,



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APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

- 1 1. (Twice Amended) A method of increasing the efficiency of transfection
2 of cycling cells sensitive to high energy electromagnetic radiation, comprising:
3 synchronizing at least 30% of said cells at a first stage of the cell cycle by
4 contacting said cells with high energy electromagnetic radiation, and
5 transfecting said cells at a second stage of the cell cycle within about one cell
6 cycle of said first stage with a nucleic acid that encodes a desired gene product.
- 1 2. A method of claim 1 wherein said high energy electromagnetic radiation
2 synchronizes cells at a stage of the cell cycle when the nuclear membrane is substantially
3 degraded.
- 1 3. A method of claim 1 wherein said high energy electromagnetic radiation
2 synchronizes cells at late S phase.
- 1 4. A method of claim 1 wherein said high energy electromagnetic radiation
2 synchronizes cells at the G₂/M phase boundary.
- 1 5. A method of claim 1 wherein said high energy electromagnetic radiation
2 synchronizes cells at a stage other than M phase, and the nucleic acid accumulates in cells that
3 have cycled to the G₂/M phase boundary.
- 1 10. (Amended) A method of claim 1 wherein said nucleic acid is [part of a]
2 fully encapsulated in a lipid-nucleic acid particle.
- 1 11. The method of claim 1 wherein said high energy electromagnetic
2 radiation is a member selected from the group consisting of Gamma rays, X-rays, and ultraviolet
3 rays[, infrared rays and microwaves].

- 1 12. The method of claim 11 wherein said high energy electromagnetic
- 2 radiation is X-rays.